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Diagnosis of natural rubber latex allergy

Robert G. Hamilton*

Division of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21224, USA

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Abstract

The accurate diagnosis of a latex-allergic individual begins with a comprehensive clinical history. Atopy, food allergies, hand dermatitis, and temporal relationships between allergic symptoms and natural rubber product exposure are risk factors that increase the suspicion of latex allergy. If symptoms are temporally delayed (hours–days) and confined to skin–latex product contact areas, Type IV hypersensitivity should be suspected and patch testing may be performed to identify activated T cells that are specific for selected rubber chemical additives. If ocular, upper and lower airway, and/or systemic allergic symptoms are observed with rapid onset (minutes) following a definable latex exposure, Type I hypersensitivity should be suspected. One or several confirmatory tests for latex-specific IgE antibody in the skin or blood may next be performed to verify a sensitized (IgE antibody positive) state. If the clinical history remains discordant with a skin test or blood test result, in vivo provocation tests may be cautiously considered for adjudication. Diagnostic methods for latex-specific IgE antibody detection in skin and blood are overviewed, with a focus on their performance, advantages, and limitations.

Keywords: Natural rubber (*Hevea brasiliensis*) latex allergy; Latex-specific IgE antibody; Skin test; Immunoassays; Radioallergosorbent test; Provocation tests

1. Introduction

Natural rubber latex allergy emerged in the 1980s as a recognized public health concern. Since this time, investigators studying the epidemiology and management of latex allergy have put forth great effort to accurately identify or diagnose individuals who are both sensitized and clinically allergic to latex. It is well established that individuals can be sensitized or IgE antibody positive to natural rubber latex proteins but remain asymptomatic or free of any clinically observable symptoms [1]. With further exposure, some individuals become symptomatic with latex allergen provocation. Correctly identifying the individuals who have become sensitized and either manifest symptoms or are at risk of clinical symptoms has been the key issue that has determined the quality of research on the topic. The goal of this paper is to examine the rationale for designing diagnostic latex reagents, the evolution of the clinically used diagnostic algorithm for assessing latex-sensitized individuals, and the performance of past and present latex reagents and methods that are used in the diagnosis of latex allergy.

2. Philosophy in the design of diagnostic latex reagents

Natural rubber latex from the *Hevea brasiliensis* tree reportedly contains more than 250 polypeptides, at least 60 of which demonstrate IgE binding properties [2]. At least 11 different *Hevea* proteins are known to elicit IgE antibody and these may be subcategorized into four families based on their known biological functions (Table 1). The individual allergens are discussed in extensive detail elsewhere in this issue [3]. The primary, secondary, and tertiary structures of these *Hevea* proteins dictate the proteins' relative solubility, size and the compactness of the overall folds [4]. Of utmost importance to this discussion, however, is the stability or ability to resist structural alternation that can readily occur as a result of extreme temperature, pH, and chemical modification during rubber product manufacturing. The relative quantities of the *Hevea* allergens released from rubber products into the environment

* Fax: +410-550-2030.
E-mail address: rhamilt@jhmi.edu.

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Table 1
Principal *Hevea brasiliensis* allergenic proteins[a]

Name	Description	Allergen group	Molecular mass (kDa)	p*I*
Hev b 1	Rubber elongation factor	1	14.6	8.45
Hev b 2	β1,3-Gluconase	3	34–36	10
Hev b 3	Prenyltransferase	1	24–27	4.8
Hev b 4	Microhelix	4	110/50	4.5
Hev b 5	Acidic protein	4	16–24	3.5
Hev b 6	Hevein protein (6.01/6.02/6.03)	2, 3	20/4.7	4.9
Hev b 7	Patatin homolog (7b/c)	2, 3	43–36	4.8
Hev b 8	*Hevea* profilin	4	14–14.2	NA
Hev b 9	*Hevea* enolase	4	51	NA
Hev b 10	Mn superoxide dismutase	4	22–26	NA
Hev b 11	Class I chitinase	3	33	NA

[a] Functional Group 1: *Facilitate biosynthesis of polyisoprene polymer*. Hev b 1 facilitates action of prenyltransferase enzymes to produce polyisoprene rubber chains. Functional Group 2: *Coagulation of latex particles*. Hevein is a lectin-like protein that binds chitin and aids in coagulation via interaction with *N*-acetyl-D-glucosamine and a 22-kDa glycoprotein receptor on the surface of rubber particles. Hev b 7 inhibits coagulation. Functional Group 3: *Pathogenesis-related proteins.* Protect plants against pathogenic microorganisms by degrading fungal cell walls and chitin exoskeletons of insects and promoting plant wound healing. Functional Group 4: *Structural proteins and housekeeping enzymes.*

dictate the routes to which genetically predisposed individuals get exposed [5]. All these factors contribute to the ultimate individual heterogeneity of the Hev b-specific IgE antibody specificity that is detected in the blood and skin of each sensitized individual. Certain *Hevea* proteins, especially Hev b 2, 5, 6, and 7b, elicit IgE antibodies in more than 50% adult health care workers and are thus classified as primary allergens [6–8]. Additionally, Hev b 1- and 3-specific IgE antibodies may be considered primary allergens for children with spina bifida. As a general rule, however, the design of diagnostic latex reagents for skin testing and detection of latex-specific IgE antibodies in the blood should be based on a principle of "all inclusiveness." In other words, all of the allergenic proteins present in *Hevea* latex should be represented in a latex skin testing extract or an allergen-containing allergosorbent used in a solid-phase immunoassay for IgE antibody [9,10]. To not include all allergens, ideally in molar excess to their levels in the skin or blood of sensitized individuals, is to ensure a less than optimal performance of the reagents and to relegate their diagnostic sensitivity and specificity to suboptimal standards.

3. Historical context of diagnostic methods

By 1992, a group of early diagnostic assays for the detection of latex-specific IgE antibody in human skin and blood were available at research centers in North America and Europe. These assays had been used to identify latex-specific T cells and latex-specific IgE antibody using sensitized basophils, skin testing, and radioallergosorbent type assays [11–14]. The diagnostic algorithm initially used in North America began with a clinical history. Individuals suspected of being allergic to latex were then evaluated with a confirmatory test for latex-specific IgE antibody in the blood, by direct provocation of the skin or upper and lower airways, and lastly, by a skin prick/puncture test [14,15]. In the United States, skin testing was last in the pecking order because of a concern for the safety of the patient. More specifically, systemic reactions including anaphylaxis with hypotension, hives, angioedema, and/or wheezing had been reported in 9 of 107 patients and 0 of 10 control subjects who underwent skin prick/puncture testing with a 24-h phosphate-buffered saline (PBS) extract of powdered latex medical gloves [15]. This high (8.4%) frequency of severe adverse reactions reported with latex puncture skin testing raised doubts in the minds of the practicing allergist about an otherwise safe skin test procedure. Puncture skin testing with other allergens was known to produce a 0.04–1.4% frequency of severe adverse reactions such as asthma and anaphylaxis [16–18]. Fortunately, with time and the use of other latex reagents, puncture skin testing with latex was shown to also be a safe procedure. Thus, the present-day diagnostic algorithm involves an initial clinical history that is followed by a diagnostic puncture skin test when a standardized latex reagent is available, serology for latex-specific IgE antibody using a documented assay method, and lastly, in vivo provocation testing.

4. Clinical history

A comprehensive clinical history is the first step in the diagnosis of latex allergy. Patient complaints often begin with a spectrum of allergic symptoms—skin and upper and lower airway symptoms—that are temporally associated with exposure to natural rubber latex products. These are discussed more in detail by Sussman et al. in this issue [19]. Health care professionals, for instance, use powdered latex medical gloves, which are known to

contain relatively high levels of latex allergens that can become airborne [20,21]. Cornstarch donning powder in powdered latex gloves is not generally considered allergenic, but it is reportedly a carrier for latex allergen [22]. Once an individual becomes sensitized (latex-specific IgE antibody positive), he or she may remain asymptomatic until subsequent latex allergen exposure induces further sensitization and overt skin, ocular, and upper and lower airway allergic symptoms [1]. The temporal association between latex exposure and allergic symptoms can be used as one important historical hallmark of latex allergy.

The suspected latex-allergic individual may be questioned about a number of issues [23]. Is the individual atopic, with seasonal rhinitis, early-onset asthma, eczema, and food allergy? Atopy, hand dermatitis, and allergies to certain foods are established risk factors associated with latex allergy. Is the individual repeatedly exposed to natural rubber products that are known to release latex allergen (e.g., medical gloves)? What are the time course and magnitude of the individual's allergic response in relationship to her or his latex allergen exposure? Reports of hand dermatitis, swelling, redness, and irritation confined to the area of skin/rubber contact that occur at 24–48 h are useful in discriminating type IV (contact dermatitis) hypersensitivity from type I (immediate-type) hypersensitivity, which occurs within minutes. Once the clinician has concluded that there is a high degree of suspicion for IgE antibody-mediated latex allergy, a confirmatory test for latex-specific IgE antibody should be performed to obtain support for this diagnosis.

5. Diagnostic confirmatory tests

The clinician has a choice of two confirmatory tests that have been extensively used clinically to identify a state of sensitization: the skin test and the blood test for latex-specific IgE antibody [10]. Detection of latex-specific IgE antibody in the skin is attractive because it is rapid, is sensitive, and involves a clinically observable and biological relevant response in the skin of the individual. However, since no FDA-approved latex skin testing extract is available in the United States, serological tests for latex-specific IgE have assumed greater importance as an alternative diagnostic confirmatory test. At last resort, in vivo provocation tests may be used, especially in cases where there is a convincingly positive clinical history but a negative skin test and/or blood test for latex-specific IgE antibody.

6. Skin testing

Physiological extracts of powdered natural rubber latex medical gloves and crude latex preparations directly from *Hevea brasiliensis* trees [12,14,15] were the earliest diagnostic skin test reagents available for use. Interpretation of results from these puncture skin tests was sometimes difficult, however, because the extracts were rarely uniform and often of poor quality and unstable, and their allergenic composition was unknown. A plan for systematic development of a latex skin testing material for human use began in the early 1990s.

One of the first studies was performed to examine the safety and relative diagnostic sensitivity and specificity of the three potential candidate latex source materials: nonammoniated latex, ammoniated latex, and extracts of powdered latex gloves [24]. In this study, adults with either a positive ($n = 78$) or negative ($n = 24$) history for adverse reactions to natural rubber latex gloves were recruited with informed consent. Nonammoniated latex, ammoniated latex, and glove extracts were normalized to 1 mg/ml total protein, and subjects underwent sequential puncture and then intradermal skin tests with all three extracts at 1–1000 μg/ml (puncture) and 1 pg/ml to 1 μg/ml (intradermal). A radioallergosorbent test (RAST) for serum IgE antibody and a two-stage latex/vinyl glove use/provocation tests were also performed to clarify the latex allergy status of 9 of the 59 subjects with a positive history for type 1 hypersensitivity to latex who had negative puncture skin and intradermal skin tests.

A number of significant observations were made from this study. First, there was the lack of large local or systemic adverse reactions, even when puncture and intradermal skin tests were simultaneously applied with all three source materials over a several-hour period. This supported the safety of the skin testing materials and methods. Second, the three diverse latex sources displayed remarkably equivalent diagnostic performance (sensitivity, specificity). This was attributed to the fact that each had been normalized to 1 mg/ml total protein. Optimal diagnostic sensitivity and specificity for all three latex source materials were achieved at 100 μg of protein/ml for skin puncture and 1 μg of protein/ml for intradermal skin tests. The nonammoniated latex appeared to be the most promising latex source material because it displayed the most reproducible interbatch potency in quality control radioallergosorbent inhibition tests [24]. It also produced the clearest banding patterns on SDS–PAGE and Western blot analysis. Variations in the observed potency of ammoniated latex and glove extracts were attributed to storage-dependent protein degradation. Since ammoniated latex was used in the manufacture of rubber products, concern has been raised as to whether the nonammoniated latex might contain allergenic proteins that are structurally different from those released by latex glove products produced with ammoniated latex. To date, there has been no empirical evidence from this study to suggest that nonammoniated latex skin testing reagents have been unable to detect latex-specific IgE antibody in the

skin of latex-allergic subjects that was detectable with ammoniated latex.

A multicenter study was subsequently performed to verify safety and define the diagnostic sensitivity and specificity of a prototype *Hevea brasiliensis* nonammoniated latex extract produced by Greer Laboratories, a licensed allergen manufacturer in Lenoir, North Carolina [23]. In this study, subjects were classified according to their clinical history as being latex-allergic (124 adults and 10 children) or non-latex-allergic (180 adults and 10 children). With consent, subjects provided a blood sample for latex-specific IgE testing. Each then underwent sequential puncture skin tests with Malaysian nonammoniated latex at 1, 100, and 1000 μg/ml latex protein using a bifurcated needle. Intradermal skin testing was not performed in this study since puncture skin testing at 100 μg/ml latex protein was shown to be equivalent to the optimal intradermal skin test results and inherently safer. A two-stage glove provocation test (see below) was performed on those individuals who had a puncture skin test result that was discordant with their clinical history.

In terms of safety, only mild reactions (mainly pruritis) were recorded in 15.1% of latex-allergic adults and 4.4% of non-latex-allergic adults as a result of skin testing. No reactions required treatment with epinephrine. These data reinforced the belief that puncture skin tests with the Greer nonammoniated latex were safe. In terms of diagnostic performance, the Greer puncture skin test reagent displayed 95% sensitivity and 100% specificity at the 100 μg/ml concentration. In later discussions, concerns were raised about a potential bias associated with the use of the glove provocation test to reassign the history status of some study subjects (e.g., those with a positive history and negative skin test). To address these potential concerns about bias of case assignment and data analysis, the skin test data were reanalyzed by an independent investigator using the initial clinical history to assign the subject's latex allergy status. Reanalysis of the multicenter study data using "intent to diagnose" and "according to protocol" statistics resulted in a diagnostic sensitivity of 70–75% (95% CI, 65–83%) when the original clinical history alone was used to define the latex allergy status of subjects. The diagnostic specificity of the Greer NAL remained essentially unchanged at 97–99% whether the initial or revised history was used to define the latex allergy status of the subject.

Other studies have been performed using a variety of skin test reagents. In 1995, Hadjiliadis et al. used unprocessed *H. brasiliensis* ammoniated latex from Bencard to perform puncture skin testing [25]. They used a wheal 3–5 mm greater than the negative control at 10 min as their criterion for a positive puncture skin test and detected a positive latex skin test prevalence of 4.2% among a hospital-based population of patients in their allergy practice. In a subsequent study, they extended these observations and showed a positive association between the size of the puncture skin test response in latex-allergic subjects and the severity of the latex-induced symptoms [26].

Ebo et al. [27] performed puncture skin test titrations with nonammoniated latex from 10^{-3} to 1 mg/ml. Using a positive skin test criterion of a >3-mm wheal diameter, they observed positive skin tests in 68% of history-positive patients. The performance of an additional intradermal skin test increased their diagnostic sensitivity to 97%. Safety was again supported by the general lack of adverse systemic reactions resulting from either puncture and/or intradermal skin testing. However, since no quality control data or comparative data with other skin testing reagents have ever been provided with this nonammoniated latex, it has never been considered a candidate for FDA licensure.

Turjanmaa et al. [28] studied the analytical performance of a diagnostic nonammoniated latex puncture skin test reagent manufactured by Stallergenes. They puncture skin tested latex-allergic ($n = 46$) and non-latex-allergic ($n = 76$) subjects who had been identified by their history. The Stallergenes reagent at 22 μg of protein/ml produced a diagnostic sensitivity (93%) and specificity (100%). In Western blot analysis, it displayed banding patterns at 14, 20, 27, 30, and 45 kDa that corresponded to known IgE-binding *Hevea* proteins (Table 1). Both the collection method and the in vitro quality control data of the two nonammoniated latex preparations produced by Greer and Stallergenes reagents suggest that they are comparable puncture skin test reagents.

Vandeplas et al. [29] evaluated the Stallergenes NAL puncture skin test reagent in 45 patients with suspected latex-related occupational asthma. In contrast to other studies, the study conclusions were strengthened by performing an inhalation challenge with powdered latex gloves and using pulmonary function test results with spirometry as their objective endpoint. The history and prick skin test both displayed a high diagnostic sensitivity (87 and 100%, respectively) in their study, but both had low diagnostic specificity (14 and 21%, respectively) when compared with the latex glove inhalation provocation challenge as the reference method for defining latex-induced asthma. The overall negative predictive value of their testing increased from 50 to 71% by combining results of the puncture skin test and history, while the positive predictive value of the combined testing modalities remained essentially unaltered at 75–76%.

At present, allergists in the United States do not have a characterized skin testing extract that they can use clinically. For this reason, either allergists prepare their own in-house latex extracts from powdered latex gloves if they wish to perform a latex skin test or they do not perform skin testing at all. Glove extracts are prepared

by soaking a glove (often the one used by a patient) for 15–30 min just prior to skin testing [15]. Glove extracts are therefore rarely assessed for potency or tested for stability. While there are rare exceptions [30], these glove extracts are of questionable quality and in limited quantity. The clinical use of glove extracts for skin testing is thus not advised because they have an unknown diagnostic accuracy and potency. A standardized latex skin testing reagent should be eventually licensed by the FDA for use in the United States.

7. Serological testing

Research serological assays for latex-specific IgE antibody detection in human serum were discussed at the 1992 international conference on sensitivity to latex products in medical devices [31–33]. Subsequently, a number of latex allergen preparations from gloves and ultracentrifuged ammoniated and nonammoniated *H. brasiliensis* latex have been used to prepare allergosorbents that bind latex-specific antibodies [34–36].

In 1994, we compared the performance of a particle-based RAST that used nonammoniated latex, ammoniated latex, and powdered latex glove extracts source latex preparations [34]. The goal of this study was to identify the optimal latex source material from which the allergosorbents could be reproducibly prepared for use in the detection of latex-specific IgE antibodies in human serum. Our working hypothesis was that proteins extracted from a latex glove or present in the C-serum of ammoniated latex might be the most relevant allergenic proteins since they should reflect the allergenic proteins that are released from medical gloves. However, unfortunately, these sources of latex proteins were known from skin testing studies [24] to sometimes vary in potency between lots. Thus, they were considered too unstable for long-term production of latex allergosorbents. Empirically, however, we observed good concordance among the percentages of sera with positive latex-specific IgE antibody levels when they were tested in RAST-type assays that used the glove, ammoniated latex, and nonammoniated latex preparations from *H. brasiliensis* Clone 600 trees. This suggested that the principal allergenic latex proteins could survive intact following immersion in ammonia and harsh glove manufacturing processes. However, due to concerns about the variability in allergen potency of glove extracts and ammoniated latex, we selected nonammoniated latex for our subsequent clinical evaluations. Our radioallergosorbent test using nonammoniated latex as the solid-phase allergen displayed interassay coefficients of variation <20% and an analytical sensitivity of 0.6 ng/ml [34]. Latex-specific IgE antibody was detected with this assay in the range 0.7–338 ng/ml in serum from two-thirds of the 33 latex-allergic subjects (66% sensitivity) and one of the 10 non-latex-allergic controls (90% specificity).

Kurup et al. [35] reported an enzyme-linked immunoassay that used glove extracts and ammoniated latex from Malaysia and India as their allergen source. They tested sera from latex-sensitized ($n = 45$) and nonsensitized ($n = 27$) subjects and observed considerable differences in reactivity of IgE antibody with the different antigens. Sera from 50% of the Finnish and 72% of the American subjects with clinical evidence of latex allergy were positive in these assays (50–70% sensitivity). Moreover, anti-latex IgE measurements were positive in only 7.4% of clinically non-latex-allergic controls, yielding a 92.6% specificity.

A novel RAST variant involved the use as the allergosorbent of a disk of latex that had been punched directly from a latex glove [36]. Unfortunately, use of the glove disk did not improve assay sensitivity, and variable nonspecific binding on the glove disk compromised the assay's specificity. So this interesting strategy for preparing a latex allergosorbent was not pursued further.

In the United States, FDA clearance is required only when the manufacturer of a latex-specific IgE antibody assay desires to sell its product commercially [37]. For this reason, there was no requirement that performance data from latex-specific IgE research assays [31,32] that were being used in federally licensed clinical laboratories be submitted to the FDA for review. The first commercial serological assay to be FDA cleared for detection of latex-specific IgE antibody was the Diagnostic Products Corporation (DPC)'s tube-based enzyme immunoassay (March 1995). This was later replaced with a microtiter plate-based immunoassay in August 1995. More recently, DPC's Immulite Assay (November 1996), Pharmacia's CAP System FEIA (December 1996), Pharmacia's UniCAP FEIA assay (July 1997), and other assays have been cleared by the FDA as being substantially equivalent in their performance to the tube-based AlaSTAT.

We compared the diagnostic performance of three FDA-cleared in vitro assays (DPC Microplate Alastat, Pharmacia CAP System FEIA, and Hycor HYTECH) for the detection of natural rubber latex-specific IgE antibody [38]. Sera from 117 clinically latex-allergic and 195 clinically nonallergic subjects were obtained from those who participated in the multicenter latex skin testing study [23]. The diagnostic sensitivity and specificity of these three FDA-cleared assays using the history and puncture skin test with the Greer experimental latex reagent as reference methods are summarized in Table 2. Importantly, the Pharmacia CAP System and DPC Alastat microplate displayed a diagnostic specificity of 97% and a 76 or 73% diagnostic sensitivity, respectively, when compared with the latex skin test. Both assays therefore misclassified approximately 25%

Table 2
Performance of FDA-cleared allergen-specific IgE serology tests[a]

Method	Subjects and mode of classification	Diagnostic sensitivity	Diagnostic specificity	Positive predictive value	Negative predictive value	Efficiency	Ref.
Pharmacia CAP System[b]	117 Hx+ 195 Hx−	75.2% (67–83%)	90.8% (87–94%)	83.0% (76–90%)	85.9% (81–91%)	84.9% (81–89%)	[38]
Pharmacia CAP System[b]	131 PST+ 181 PST−	76.3% (69–84%)	96.7% (94–99%)	94.3% (90–99%)	85.0% (80–90%)	88.1% (85–92%)	[38]
Pharmacia CAP System[c]	83 latex-allergic 60 non-latex-allergic	79.5%	90%	91.7%	76.1%	NA	[39]
DPC AlaSTAT[b]	117 Hx+ 195 Hx−	78.6% (71–86%)	95.4% (92–98%)	91.1% (86–97%)	91.1% (84–93%)	89.1% (86–93%)	[38]
DPC AlaSTAT[b]	131 PST+ 181 PST−	73.3% (66–80%)	97.2% (95–99%)	95.0% (91–99%)	83.4% (78–88%)	87.2% (83–91%)	[38]
DPC AlaSTAT[c]	83 latex-allergic 60 non-latex-allergic	74.7%	91.7%	92.5%	72.4%	NA	[39]
Hycor HyTECH[b]	117 Hx+ 194 Hx−	89.8% (84–95%)	67.5% (61–74%)	62.5% (55–70%)	91.6% (87–92%)	75.9% (71–81%)	[38]
Hycor HyTECH[b]	131 PST+ 180 PST−	91.6% (87–96%)	73.3% (67–80%)	71.4% (65–78%)	92.3% (88–97%)	81.0% (77–85%)	[38]

[a] *Diagnostic sensitivity*: frequency of a positive latex-specific IgE test when the individual has latex allergy. *Diagnostic specificity*: frequency of a negative latex-specific IgE test when the individual does not have latex allergy. *Positive predictive value*: percentage of all positive results that aretrue positives or the frequency of latex allergy in patients with a positive latex-specific IgE antibody test. *Negative predictive value*: percentage of all negative IgE anti-latex results that are true negatives or the frequency of no latex allergy in patients with a negative latex-specific IgE antibody test. *Efficiency*: percentage of latex-allergic patients correctly classified by the latex-specific IgE test as having or not having latex allergy. NA, Not available; Hx+, positive for latex allergy history; PST+, positive with latex puncture skin test.

[b] Mean results (95% confidence limits) are provided using history or puncture skin test with an investigational Greer skin test reagent to qualify latex-allergic and nonallergic cases.

[c] Mean results obtained using a 0.35 kUa/liter cutoff as positive. Subjects were classified as latex-allergic or non-latex-allergic by history and puncture skin test using a home brew natural rubber latex extract. NA, Not available; Hx+, positive for latex allergy history; PST+, positive with latex puncture skin test.

of latex-sensitized cases as falsely negative for latex-specific IgE antibody. In contrast, the HYTECH assay produced 27% false-positive results when compared with the skin test. In a separate study of performance [39], the CAP System and Alastat assays were compared using a different set of sera from 83 latex-allergic and 60 non-allergic subjects. The diagnostic sensitivity and specificity displayed by both assays were analogous to those reported earlier (Table 2): (sensitivity, 79.5 and 73.8%; and specificity, 90.2 and 91.7%, for the CAP and AlaSTAT, respectively).

More recently, evidence has been presented that the low diagnostic sensitivity in the CAP and AlaSTAT are a result of poorly represented and/or denatured allergens (e.g., Hev b 5) in their allergen-containing reagents [40]. In another study [41], latex allergosorbents used in the CAP and Alastat assays appeared to be missing immunoreactive allergenic epitopes from multiple latex allergens (Hev b 2, 4, 5, 6, and 7b). It was not clear whether there is a total absence of these complete immunoreactive Hev b allergens in the commercial diagnostic latex reagents or just denatured epitopes.

Biagini et al. have performed reproducibility studies in which they have observed significant imprecision of commercial latex-specific IgE antibody assays at levels approaching their reported positive cutoff values (0.35–0.45 kIU/liter) [42]. Of concern were the 35% of sera that produced discordant positive or negative mismatched discrepancies with repeat testing in the same assays. This high variation near the positive threshold with the DPC AlaSTAT assay has also been reported by a second group, which concluded that latex-specific IgE results in the range 0.35–0.7 kIU/liter should be reported as equivocal [43]. The data from both studies support the importance of duplicate or repeat testing when a latex-specific IgE result falls into this low positive range.

8. In vivo provocation tests

Demonstration of a direct temporal association between exposure to a known latex allergen source and the induction of allergic symptoms has provided investigators with an independent confirmation of latex sensitization and support for the clinical diagnosis of latex allergy. The greatest utility of provocation testing, however, has been as an arbitrator in clarifying the actual latex sensitivity status of individuals who have negative latex-specific IgE antibody serology and/or skin test results while also having a convincing positive latex allergy history [44]. The difficulty with interpreting provocation test results, however, has been the variation among reported protocols in terms of the latex allergen sources used (glove source), the application or exposure

time duration of the subject, and the method used in scoring induced symptom endpoints. Provocation tests can be subdivided into several groups depending primarily on the mode of latex allergen exposure.

8.1. Natural environmental exposure

The most fundamental form of latex allergen provocation involves monitoring patients for allergic symptoms and pulmonary function when they are working in a latex allergen-containing environment. It has been difficult to accurately quantify the level of latex allergen in the patient's environment and more importantly, to objectively quantify latex-induced allergic symptom endpoints which are often subjective in nature (e.g., itching). Marcos et al. [45] used serial inhalations of an aqueous surgical glove extract to induce a latex allergen exposure. Using spirometry, they observed a decline in pulmonary function of one latex allergic-patient but not in two nonallergic controls. The conclusions of their study were limited by the extremely small sample size. The paper, however, demonstrates one possible approach for performing a latex provocation test.

In their study, Pisati et al. [46] had individuals inhale serial dilutions of aqueous extracts from powdered and nonpowdered latex rubber gloves and control cornstarch that had been nebulized into an exposure room. They observed a decrease in spirometry-based pulmonary function measurements with all four latex-allergic subjects after exposure to powdered latex surgical glove extracts, in two subjects following exposure to nonpowdered latex surgical glove extracts, and in no subjects after exposure to an extract of untreated cornstarch powder. The weakness of this study again resides in the small sample size of the study population.

8.2. Glove use tests

Powdered latex medical gloves have been shown to be a potent source of natural rubber latex allergen [47]. Heese et al. [48] performed a different type of glove exposure test to evaluate history-positive individuals with equivocal or unsatisfactory puncture skin tests and/or patch tests. Subjects were asked to wear gloves for 2 h daily on 3 consecutive days and they were monitored for allergic symptoms. Since the source and type (powdered, relative allergen content) of gloves and the endpoint reaction scoring method were not specified, it has been difficult for others to replicate their work. In a different study, Jaeger et al. [36] described a glove exposure test in which powdered latex gloves (one untreated and the second washed for 48 h in saline) were concurrently placed on the left and right hands of the subject. Differences were noted in symptoms and skin reactions between the hands in some subjects that received the unwashed powdered latex glove and washed control latex glove. Moreover, identical skin reactions were observed on both hands of four of the latex-allergic patients. Some even had stronger reactions on the hand with the washed control glove, which theoretically had lower levels of allergen. The authors concluded that the two-hand provocation test was not useful since it could not distinguish between localized skin reactions induced by the unwashed test glove and washed control glove.

Attempts have been made by other investigators to grade the latex allergen exposure by sequentially applying a finger, patch, and intact glove to the hand of sensitized individuals. The two major limitations in these studies have been the subjective nature of the endpoint measurements and the difficulty in masking of the procedure. To make matters worse, the allergenic content of latex gloves is often variable, even between lots of gloves from the same manufacturer, so it has been virtually impossible to replicate exposures, sometimes even within a study between subjects. The reproducibility of allergen exposures through the skin has also been difficult because the intact skin effectively limits the transfer and adsorption of latex allergen. One approach to resolving this issue of skin exposure has been to modify the glove provocation procedure by adding a puncture of the skin just prior to applying the glove [49]. The procedure involves donning a vapor mask and goggles to prevent inhalation and ocular exposure and then washing but not drying the subject's hands. The skin is then punctured through saline with a bifurcated needle in three places on the volar aspect of both hands. Both hands then receive high-allergen-powdered latex gloves (Baxter-Allegiance Triflex), with one hand being first covered with a synthetic glove or protective glove liner (Dermapor) to serve as a negative control. Gentle circular pressure is then applied by rubbing over the puncture sites 50 times. The subject is monitored for 30 min for any evidence of skin and respiratory symptoms. In the study of 17 latex skin test- and RAST-positive subjects, all experienced localized pruritis with measurable wheals and erythema at the puncture sites on the latex glove-exposed hand but not the protected control hand. Some have viewed this as simply a modified skin test. The puncture event serves to increase the relative sensitivity of the glove use test by abrading the skin and facilitating latex allergen transport into the skin. Concerns, however, remain, principally among these being the masking of the procedure, quantifying and reproducing a defined latex allergen exposure, and assessing relevant conjunctival and respiratory responses.

A second stage was added to this "modified glove provocation" to evaluate the respiratory component as well as cutaneous reactions [49]. Once the mask and goggles are removed, the subject is directed to blow up a new powdered latex glove like a balloon and expel its

contents slowly into their own face. This gross exposure is repeated twice and the subject is then monitored for 60 min for changes in measured peak expiratory flow rates and reported symptoms. This two-stage glove provocation procedure was used in the multicenter skin testing study [23] to adjudicate discordant latex allergy histories and skin test results. Of the 158 latex history positive subjects in the study, 25 had negative latex skin tests. Of these, 24 had negative two-stage challenges and one had a positive two-stage inhalation challenge. Moreover, 5 of 180 subjects with a negative history of latex allergy had a positive latex skin test and all 5 of these individuals had a negative two-stage provocation test. These data can be used to identify individuals who are sensitized but asymptomatic and those who may have a false-positive skin test. The second stage of the provocation procedure is also difficult to mask and quantify the actual latex allergen exposure.

In a study conducted by Niggemann et al. [50] the modified glove provocation procedure with a glove wearing and sequential glove inflation procedure was used to evaluate latex allergy in children with spina bifida. The children were asked to remove their mouth from the inflated glove while inhaling to prevent inspiration of latex particles. For the 11 children who were too small to cooperate, the glove was inverted inside out and rubbed gently on the lips for 1 min. A positive provocation test with localized urticaria, in some cases including swelling of the lips, was observed in 55 of 159 challenged children (62.5% of sensitized patients).

In an attempt to deliver a more controlled exposure, Laoprasert et al. [51] exposed subjects in an inhalation chamber. They attempted to increase the levels of latex allergen progressively by performing seven sequential challenges which involving donning and discarding a vinyl, low-latex-allergen (powder-free) glove and five sets of powdered latex gloves with increasingly higher latex allergen levels. After each exposure, symptom scores and spirometry (FEV_1) were monitored for 60 min. A laminar flow helmet was worn by the subject during all challenges. Only during the first two challenges with the powdered high-latex-allergen gloves did investigators insert a HEPA filter in-line as a control to stop all particles from being inhaled. The investigators concluded that latex allergen levels inside and outside the helmet were not significantly different with the use of the vinyl and powder-free gloves. Symptom scores increased and FEV_1 performance declined with the third to fifth powdered latex glove challenges following removal of the HEPA filter. The graded glove challenge in theory provided a more controlled and safer exposure to latex allergen from powdered latex gloves. Strengths of the study were the quantitative measures of latex allergen exposure and semiquantitative symptom endpoints. Its limitations were the continued difficulty in masking the provocation and the need for an expensive environmental containment chamber that is in limited supply.

8.3. Nasal challenge

Palczynski et al. [52] performed a single-blind placebo-controlled latex nasal provocation study on nurses with bronchial asthma and/or rhinitis. Their goal was to investigate the cause of health care workers' occupationally induced rhinitis. They used pooled nasal washings that were collected following topical administration of latex allergen or placebo saline. Cellular responses and changes in protein and ECP levels were measured and the administration of latex but not saline in the nose was shown to induced a significant increase in eosinophil and basophil numbers, albumin-to-total protein ratio, and ECP levels. This occurred only in the natural rubber latex puncture skin test-positive subjects. The reported latex nasal challenge test is an interesting research tool, but it is unlikely to be used widely for clinical assessment of patients due to the special clinical and laboratory requirements for performing nasal lavage and the cellular studies.

8.4. Hooded exposure chamber

In an attempt to increase safety and provide more controlled progressive exposure to the upper and lower airway and conjunctiva, Kurtz et al. [53] described a device called the hooded exposure chamber (HEC). In this system, latex allergen cornstarch that was isolated from powdered latex gloves was diluted serially with untreated cornstarch. A cloud of latex allergen-associated cornstarch particles was produced with an impinger device and it was directed into the hood with an air pump. The subject wore the hood over the head and breathed normally as the particulate levels changed each 18 min from no latex allergen (untreated cornstarch as a negative control) to higher levels of latex allergen-associated cornstarch particles. The number and particle size distribution flowing into the hood were monitored with an on-line particle laser counter. Chest and rhinoconjunctival symptoms were scored using a previously validated system [54]. Peak expiratory flow rates were also monitored as an endpoint. The advantages of the HEC system were reported to be the possibility of masking, an inherent safety that stemmed from its graded aeroallergen exposure, and the recording of quantitative endpoint information. The need for specialized equipment and laboratory space, the variable potency of the latex allergen-associated cornstarch preparations from different sources of powdered latex gloves, and the labor intensive nature of challenging with a series of aeroallergen concentrations were cited as limitations of the procedure.

9. Concluding remarks

The referring physician has the ultimate responsibility of ensuring quality diagnostic testing for their patients [55]. When the initial clinical history is convincing for latex allergy, a confirmatory latex-specific IgE antibody test should be performed to ensure that there is credible evidence for sensitization. If a puncture skin test is performed, then the latex reagents and procedures need to be validated and traceable to a reference material such as the nonammoniated latex (E8) produced by the FDA. In the absence of a licensed skin testing reagent, anti-latex IgE may be detected with serological assays. In this case, a federally certified laboratory should be selected to perform the testing and the assay methods that are used to measure IgE antibody should either be well documented with in-house data or cleared by FDA. One mark of excellence for clinical laboratories is federal certification by the Clinical Laboratory Improvement Act of 1988 (CLIA-88). Moreover, the clinical laboratory should have good performance in an interlaboratory performance survey such as the diagnostic allergy SE Survey conducted by the College of American Pathologists. Only at last resort, should in vivo provocation tests be performed since these procedures can be unsafe because of uncontrolled latex allergen exposures. One circumstance where provocation testing may be warranted is in the evaluation of the patient who has a strongly positive clinical history for latex allergy but a negative skin test and/or serology. In these cases, safety should be maximized with graded allergen exposures and the use of measurable endpoints for defining the objective outcomes of the provocation test.

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